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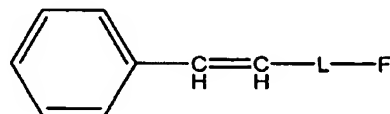
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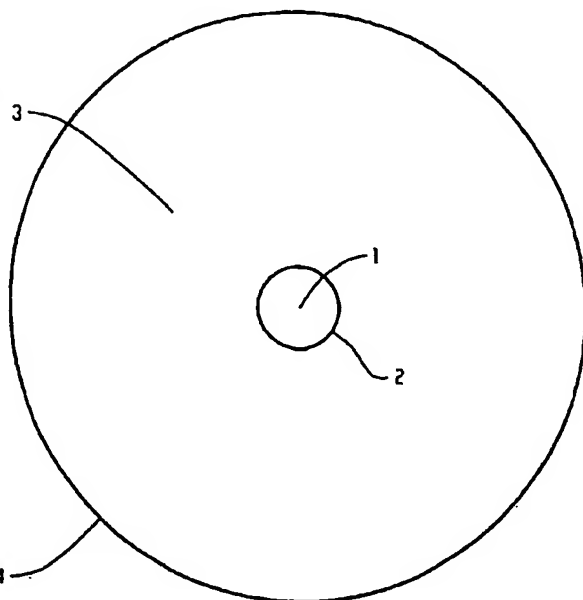
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(54) Title: **FLUORESCENT NANOPARTICLES**



(I)

(57) Abstract: A fluorescent nanoparticle includes a core comprising an alkenylbenzene; an intermediate layer, an outer shell layer, and a fluorescent portion. The fluorescent portion includes a structure represented by the following formula: (I), wherein L is a direct bond or a linker group, and F is any fluorescent moiety. The fluorescent portion is located in at least one of the following locations: the core, the intermediate layer, or the shell layer of the nanoparticle. Methods for making the fluorescent nanoparticle are also described.



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FLUORESCENT NANOPARTICLES

FIELD

[0001] The technology disclosed herein is generally related to fluorescent nanoparticles. More particularly, it relates to a fluorescent nanoparticle comprising a core, an intermediate layer, and a shell. This disclosure also provides a method of making the fluorescent nanoparticles.

BACKGROUND

[0002] Fluorescent microparticles may be prepared by several practical methods from a variety of polymerizable monomers, including styrenes, dienes, acrylates and unsaturated chlorides, esters, acetates, amides and alcohols. For example, U.S. Patent 4,326,008 to Rembaum discloses fluorescent microspheres obtained by copolymerizing an acrylic monomer containing a covalent bonding group such as hydroxyl, amine, or carboxyl with a fluorescent co-monomer such as dansyl allyl amine. U.S. Patent 5,194,300 to Cheung and U.S. Patent 4,774,189 to Schwartz disclose fluorescent microspheres that are coated by covalently attaching to their surface one or more fluorescent dyes. U.S. Patent 5,073,498 to Schwartz and U.S. Patent 4,717,655 to Fulwyler disclose fluorescent dyes added during particle polymerization process. In *Uniform Latex Particles*; Seragen Diagnostics Inc. 1984, p. 40, L. B. Bangs describes a method of internally embedding or diffusing a dye after particles have been already polymerized. U.S. Patent 5,723,218 to Haugland et al. discloses diffusely dyeing microparticles with one or more dipyrrometheneboron difluoride dyes.

[0003] Fluorescent particles to which biological molecules have been attached have been used for immunoassays, as described, for example, in U.S. Patent 4,808,524 to Snyder et al.; as labels for cell surface antigens, as described, for example, in Jett, Keller, Martin, Nguyen, & Saunders, *Ultrasensitive Molecular-Level Flow Cytometry*, in FLOW CYTOMETRY AND SORTING, p. 381, 2nd ed., Wiley-Liss Inc., N.Y. 1990; and as tracers to study cellular metabolic processes, as described, for example, in Hook & Odeyale, *Confocal Scanning Fluorescence Microscopy: A New Method for Phagocytosis Research*, J. LEUKOCYTE BIOL. 45: 277 (1989).

[0004] Particles based on micelle formation are also known, for example, U.S. Patents 6,437,050, 6,689,469, 6,956,084, 7,112,369, which are hereby incorporated by reference in their entirety. These patents disclose the method of making styrene-core and butadiene-shell micelle particles. Related publications include "*Dendrimers and Dendrons, Concept, Synthesis, Application*", edited by Newkome G.R, Wiley-VCH, 2001; and "*Synthesis, Functionalization and Surface Treatment of Nanoparticles*", edited by Baraton M-I, ASP (Am. Sci. Pub.), Stevenson Ranch, California, 2003.

[0005] Over the past several years, polymer nanoparticles have also attracted increased attention not only in the technical fields such as catalysis, combinatorial chemistry, protein supports, magnets, and photonics, but also in the manufacture of rubber products such as tires. For example, nanoparticles can modify rubbers by uniformly dispersing throughout a host rubber composition as discrete particles. The physical properties of rubber such as moldability and tenacity can often be improved through such modifications.

[0006] The production and use of fluorescent labels in medicine and biology have grown rapidly and have been very profitable in the market. The availability of a new class of fluorescent markers offering clearly improved performance and safety is a strategic interest for this market. Today, biologists employing fluorescent techniques rely on dye molecules that have serious drawbacks. Particularly, many of these dye molecules are carcinogenic. Therefore there is a need for a safer, better performing material for use in the fluorescent/bio-optical market.

SUMMARY

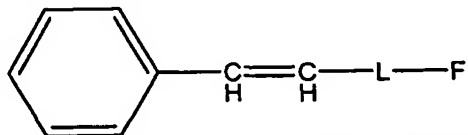
[0007] A new class of fluorescent nanoparticles, and a method for their preparation is described and claimed.

[0008] As depicted in the example shown in Fig. 1, the nanoparticles described herein are each made up of a group or a collection of several polymer chains that are organized around a center 1. The polymer chains are linked together by a core formed from dialkenylbenzene(s). The polymer chains extend from the core 2 outwardly to form an intermediate layer 3. The intermediate layer 3 includes the portions of the polymers that are not at the outer terminal end of the polymers (*i.e.*, the intermediate layer includes monomer units that are not in the shell 4). It should

be understood that the intermediate layer is not limited to a single monomer unit in each polymer chain, but may include several monomer units. Additionally, the intermediate layer may be separated into sublayers, and the sublayers may include blocks of various homopolymer or copolymer. For example a sublayer may include a block of randomized styrene-butadiene copolymer or a homopolymer such as polyisoprene or polystyrene. A shell layer or shell 4, is comprised of the monomer units or functionally or non-functionally initiated polymer chain heads at the outer terminal ends of each polymer. The shell layer 4 is the outermost portion of the nanoparticle.

[0009] The living polymers form micelles due to the aggregation of ionic chain ends and the chemical interactions of the hydrophobic polymer chains in hydrocarbon solvent. When the alkenylbenzene is added, the micelles become crosslinked and the stable nanoparticle is formed.

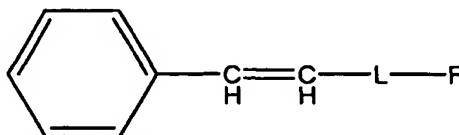
[0010] In one example, a fluorescent nanoparticle comprises (1) a core made from alkenylbenzene; (2) an intermediate layer; (3) a shell layer comprising the outer surface of the nanoparticle; and (4) a fluorescent portion that arises from the addition of a corresponding monomer with a structure represented by the following formula:



[0011] where L is a direct bond or any suitable divalent group, and F is any fluorescent moiety. The fluorescent portion is located in at least one of the following locations: the core, the intermediate layer, or the shell layer of the nanoparticle.

[0012] An example method of preparing such fluorescent nanoparticles includes: (i) preparing a living polymer with a fluorescent portion by a step selected from the group consisting of: (a) copolymerizing a fluorescent monomer with a monomer or monomers; (b) polymerizing a monomer or monomers with a fluorescent initiator; and (c) polymerizing a monomer to produce a living polymer, and subsequently adding a fluorescent monomer to the living polymer to create a fluorescent block; (ii) adding a crosslinking agent; and (iii) quenching the ionic chain ends with a proton source. After (i) but before (ii), the ionic chain ends of the polymers with fluorescent portions aggregate into micelles. The addition of the crosslinking agent causes the nanoparticle to form by producing a crosslinked core.

[0013] In yet another example, a fluorescent nanoparticle includes a core, an intermediate layer, and a shell layer. The intermediate and shell layers include ionic chain ends that extend from the intermediate layer into the core. The shell is the outermost layer of the nanoparticle. The core includes alkenylbenzene monomer units that have crosslinked the ionic chain ends of the intermediate layer. The alkenylbenzene monomers may have the same structure or may be a mixture of two or more different structures. A fluorescent portion is located in at least one of the intermediate or shell layers, or at the core. The fluorescent portion includes at least one fluorescent monomer with a structure represented by the following formula:



where L is a direct bond or any suitable divalent group, and F is a fluorescent moiety. The fluorescent monomer may be located at the core, the intermediate layer, or the shell layer.

[0014] The fluorescent nanoparticles can be used in rubber compositions.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] Fig. 1 is an example diagram of a nanoparticle;

[0016] Fig. 2 is a transmission electron microscopy (TEM) photograph of fluorescent nanoparticles; and

[0017] Fig. 3 is the microscopy picture of a film containing the fluorescent nanoparticles.

DETAILED DESCRIPTION

[0018] An exemplary fluorescent nanoparticle comprises a core including crosslinked alkenylbenzene monomers, an intermediate layer that includes polymer chains, and an outer shell layer that includes the head of the polymer chains. A fluorescent portion is located along the polymer chain in the intermediate layer, the shell, or at the core. It should be understood that the intermediate layer may have various thicknesses, *i.e.* the polymers may include one or many monomers. Preferably, the nanoparticle is less than 200 nm in diameter (expressed as a mean

average diameter), more preferably less than about 100 nm, and most preferably less than about 50 nm. The nanoparticles are preferably spherical, though shape defects are acceptable, provided the nanoparticles generally retain their discrete nature with little or no polymerization between particles.

[0019] The fluorescent nanoparticles can be copolymerized in several ways. In one example, one or more monomers are polymerized with an initiator such as butyl lithium. The resulting ionic chain ends self-assemble into micelles around a center to form an aggregate core, while the hydrophobic polymer chains radiate out away from the ionic chain ends. A crosslinking agent, such as divinylbenzene (DVB), is then added along with a fluorescent monomer (or optionally the fluorescent monomer can be added in a separate step). Acceptable crosslinking agents include di- or tri-vinyl-substituted aromatic hydrocarbons. Crosslinking agents which are at least bifunctional, wherein the two functional groups are capable of reacting with vinyl-substituted aromatic hydrocarbon monomers are also acceptable. The ionic chain ends within the aggregate core randomly react with both the DVB and fluorescent monomer to yield a crosslinked core containing fluorescent moieties. A proton source is used to quench the living polymer chains. Suitable proton sources are well known to those of skill in the art and include, but are not limited to, alcohols such as isopropanol.

[0020] In another example, one or more monomers are polymerized using a fluorescent initiator. The fluorescent initiator can be formed from a fluorescent monomer and an initiator such as butyl lithium. The resulting polymers have a fluorescent portion at one end. In a hydrocarbon solvent, the ionic chain ends aggregate into a micelle with the fluorescent portion on the outer surface. Then a crosslinking agent, such as DVB, is added to crosslink portions of the ionic chain ends of the micelle, thereby forming and stabilizing the core of the nanoparticle. A proton source is used to quench the living polymer chains.

[0021] In another example, one or more monomers and at least one fluorescent monomer are copolymerized using an initiator such as butyl lithium. The resultant living copolymers have a fluorescent portion within the chains. The ionic chain ends then self-assemble into micelles in a hydrocarbon solvent. A crosslinking agent, such as DVB, is added to crosslink portions of the ionic chain ends of the micelle, thereby

forming and stabilizing the core of the nanoparticle. A proton source is used to quench the living polymer chains.

[0022] In another example, one or more monomers are polymerized using an initiator such as butyl lithium to a desired degree of polymerization. The resulting polymers are then copolymerized with one or more fluorescent monomers. This yields living copolymer chains with fluorescent portions within the chain. The living copolymer chains then self-assemble into micelles in a hydrocarbon solvent. A crosslinking agent, such as DVB, is added to crosslink portions of the ionic chain ends within the micelle, thereby forming and stabilizing the core of the nanoparticle. A proton source is used to quench the living polymer chains.

[0023] In variations of the above exemplary nanoparticle assembly methods, additional monomers can be copolymerized with the monomer, yielding various copolymers. Furthermore, the fluorescent monomer can be added at various stages in the copolymerization so as to control where in the polymer chain the fluorescent monomer is located.

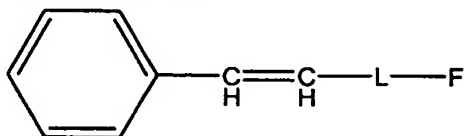
[0024] Examples of the types of monomers that may be used to prepare the polymer chains of the nanoparticles include: styrene, *t*-butyl styrene, butadiene, isoprene, copolymers of a combination of these, or derivatives thereof. Mixtures of different polymers and copolymers are also possible in a single nanoparticle.

[0025] An exemplary fluorescent nanoparticle synthesis method comprises a multi-stage anionic polymerization. Multi-stage anionic polymerizations have been conducted to prepare block-copolymers, for example in U.S. Pat. No. 4,386,125, which is incorporated herein by reference.

[0026] A liquid hydrocarbon medium can function as the solvent, and may be selected from any suitable aliphatic hydrocarbon, alicyclic hydrocarbon, or mixture thereof, with a proviso that it exists in liquid state during the preparation of the nanoparticles. Exemplary aliphatic hydrocarbons include, but are not limited to, pentane, isopentane, 2,2 dimethyl-butane, hexane, heptane, octane, nonane, decane, and the like. Exemplary alicyclic hydrocarbons include, but are not limited to, cyclopentane, methyl cyclopentane, cyclohexane, methyl cyclopentane, cycloheptane, cyclooctane, cyclononane, cyclodecane, and the like. Generally, aromatic hydrocarbons and polar solvents are not preferred as the liquid medium. In

exemplified embodiments, the liquid hydrocarbon medium comprises hexane or cyclohexane.

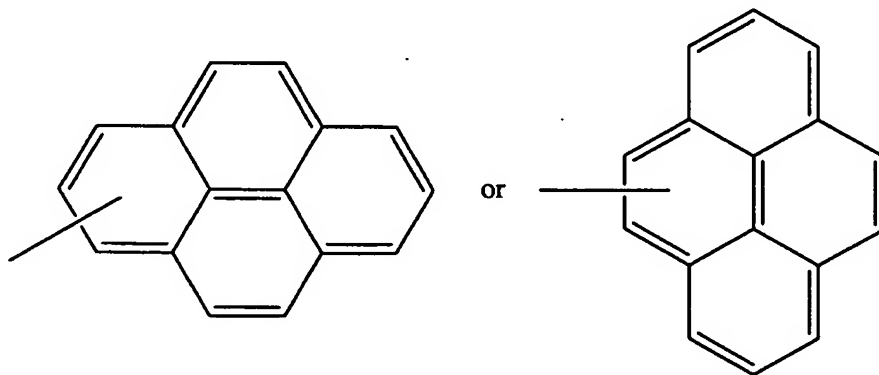
[0027] In one example, the fluorescent nanoparticles are formed from polymers having a poly(alkyl-substituted styrene) block and a polymer block of fluorescent monomers having a structure represented by the formula shown below:



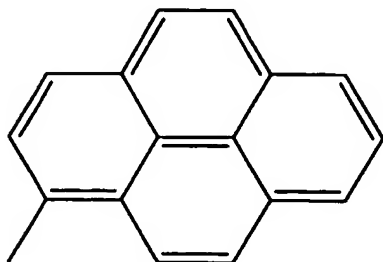
in which L is a direct bond or any suitable divalent group, and F is any fluorescent moiety.

[0028] For example, the fluorescent moiety F may be selected from the group consisting of perylene, phenanthrene, anthracene, naphthalene, pyrene, chrysene, naphthacene, and combinations thereof.

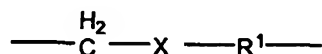
[0029] In one example, the -F group has a structure represented by the formula as shown below (pyrene):



such as:

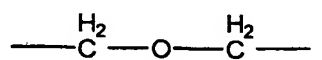


[0030] The -L- group may be just a direct bond or any suitable divalent group, for example, methylene, ethylene, and propylene group. Preferably, the -L- group has a structure represented by the formula as shown below:

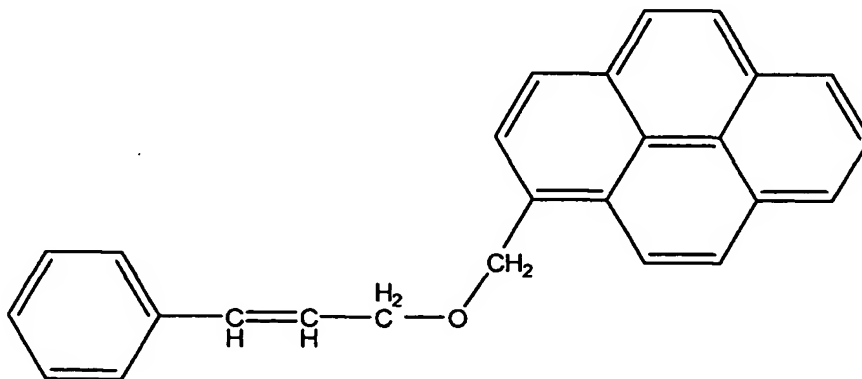


in which X comprises a heteroatom such as O, S, P(R²), Si(R²)₂, Si(OR²)₂ (where R² is as defined below), and N (where N can be substituted such that the -L- group contains a tertiary amino group); and R¹ is a straight or branched C₁-C₈ alkylene group.

[0031] In an example, the -L- group has a structure represented by the formula as shown below:

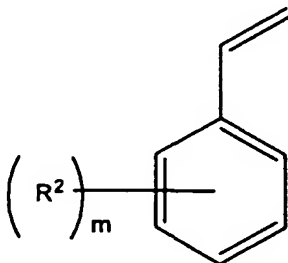


[0032] The fluorescent monomer may have, for example, a structure represented by the formula as shown below:



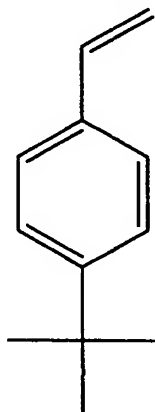
[0033] The fluorescent block may also optionally further comprise other monomers.

[0034] An example alkyl-substituted styrene block monomer of the example polymer may have a structure represented by the formula shown below:



in which m is an integer and $1 \leq m \leq 5$, preferably m is 1 or 2; and R^2 may be selected from saturated or unsaturated, substituted or unsubstituted, straight or branched, cyclic or acyclic C_3 - C_8 alkyl groups.

[0035] Another exemplary alkyl-substituted styrene monomer comprises *tert*-butyl styrene (TbST) such as *t*-butyl styrene as shown below:



[0036] It is believed that the alkyl group in the alkyl-substituted styrene monomer lowers the overall solubility of the resulting living polymer in a selected liquid hydrocarbon medium thereby facilitating micelle self-assembly and nanoparticle formation.

[0037] In one example, the alkyl-substituted styrene monomer may be copolymerized with any suitable fluorescent comonomers; and as a result, the later formed nanoparticles will have a fluorescent intermediate later. Fluorescent comonomers for this purpose include, but are not limited to cinnamyl-O-CH₂-pyrene. An exemplary polymerization of alkyl-substituted styrene monomers into a poly(alkyl-substituted styrene) block is initiated *via* addition of anionic initiators that are known in the art. For example, the anionic initiator can be selected from any known organolithium compounds. Suitable organolithium compounds are represented by the formula as shown below:



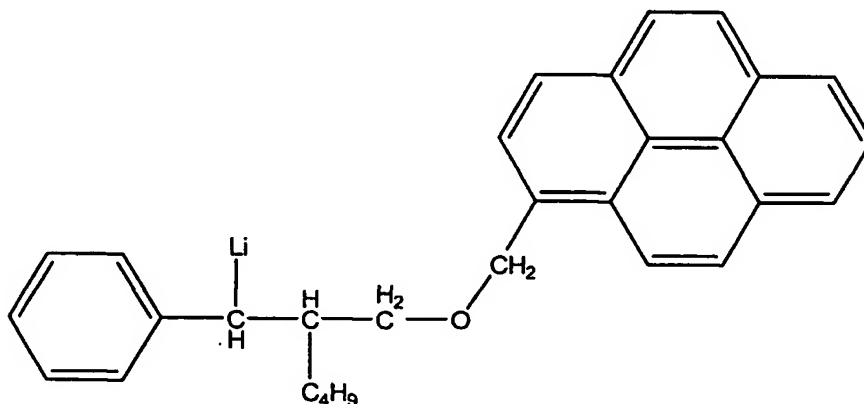
wherein R is a hydrocarbyl group having 1 to x valence(s). R generally contains 1 to 20, preferably 2-8, carbon atoms per R group, and x is an integer of 1-4. Typically, x is 1, and the R group includes aliphatic groups and cycloaliphatic groups, such as alkyl, cycloalkyl, cycloalkylalkyl, alkylcycloalkyl, alkenyl, as well as aryl and alkylaryl groups.

[0038] Specific examples of R groups include, but are not limited to, alkyls such as methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, *t*-butyl, *n*-amyl, isoamyl, *n*-hexyl, *n*-octyl, *n*-decyl, and the like; cycloalkyls and alkylcycloalkyl such as cyclopentyl, cyclohexyl, 2,2,1-bicycloheptyl, methylcyclopentyl, dimethylcyclopentyl, ethylcyclopentyl, methylcyclohexyl, dimethylcyclohexyl, ethylcyclohexyl, isopropylcyclohexyl, 4-butylcyclohexyl, and the like; cycloalkylalkyls such as cyclopentyl-methyl, cyclohexyl-ethyl, cyclopentyl-ethyl, methyl-cyclopentylethyl, 4-cyclohexylbutyl, and the like.

[0039] In selected examples, *n*-butyllithium, *sec*-butyllithium, *tert*-butyllithium, or a mixture thereof are used to initiate the polymerization of alkyl-substituted styrene monomers into a poly(alkyl-substituted styrene) block.

[0040] In one example, a fluorescent initiator may be used to initiate the polymerization of alkyl-substituted styrene monomers; and as a result, the later formed nanoparticles will have a fluorescent surface.

[0041] Examples of suitable fluorescent initiator include, but are not limited to, the following lithium compound:



[0042] Other examples of suitable fluorescent initiators may be obtained as taught in U.S. Published Application No. 2006/0036050, the entirety of which is incorporated herein by reference.

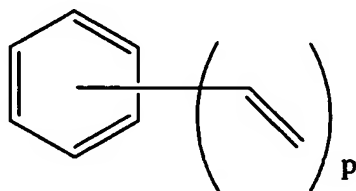
[0043] The polymerization of alkyl-substituted styrene monomers into a poly(alkyl-substituted styrene) block may last until a predetermined degree of polymerization is obtained. The degree of polymerization may be selected for particular applications. For example, a predetermined degree of polymerization of the poly(alkyl-substituted styrene) block may be broadly within the range of from about 1 to about 50,

preferably within the range of from about 1 to about 25, more preferably within the range of from about 1 to about 10, and most preferably within the range of from about 1 to about 5.

[0044] The living polymer block that contains one or more fluorescent monomers may be copolymerized or crosslinked with a multiple vinyl-substituted aromatic hydrocarbon to form the desired fluorescent nanoparticles. The fluorescent nanoparticles preferably retain their discrete nature with little or no polymerization between each other. In an example embodiment, the fluorescent nanoparticles are substantially monodisperse and uniform in shape.

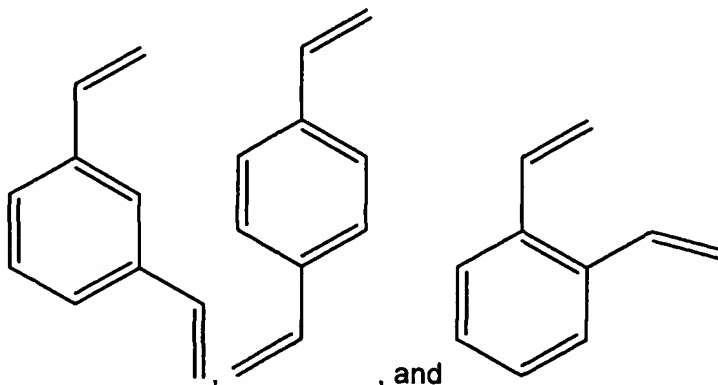
[0045] In another example, a mixture of multiple vinyl-substituted aromatic hydrocarbon and fluorescent monomer may be used to copolymerize with the poly(alkyl-substituted styrene) block, thus producing a crosslinked fluorescent core.

[0046] An exemplary multiple vinyl-substituted aromatic hydrocarbon has a formula as shown below:



in which p is an integer and $2 \leq p \leq 6$, preferably, p is 2 or 3, more preferably p is 2, i.e. divinylbenzene (DVB).

[0047] In certain examples, the divinylbenzene may be selected from any one of the following isomers or any combination thereof:



[0048] Consequently, the fluorescent nanoparticles are formed from the micelles with a core including crosslinked alkyl-substituted styrene blocks and an intermediate layer including fluorescent blocks.

[0049] The polymerization reactions used to prepare the fluorescent nanoparticles may be terminated with a terminating agent. Suitable terminating agents are known to those skilled in the art and include, but are not limited to, alcohols such as methanol, ethanol, propanol, and isopropanol.

[0050] In embodiments, the molecular weight (grams/mole) of the fluorescent nanoparticles may be broadly within the range of from about 50,000 to about 100 million, preferably within the range of from about 100,000 to about 10 million.

[0051] Various rubber articles may be manufactured from the composition as described *supra*. References for this purpose may be made to, for example, U.S. Patent No. 6,875,818, which is herein incorporated by reference.

[0052] In one example application, a composition including the fluorescent nanoparticles discussed herein may be sprayed or coated on a tire sidewall. The fluorescent property of the nanoparticles may function to improve traffic safety at night by increasing the visibility of the tires and the vehicle. Biological applications are also envisioned.

[0053] The following examples are included to provide additional guidance to those skilled in the art in practicing the claimed invention. The examples provided are merely representative of the work that contributes to the teaching of the present application. Accordingly, these examples are not intended to limit the invention, as defined in the appended claims, in any manner.

EXAMPLES

Example 1: Preparation of Cinnamyl-O-CH₂-pyrene Fluorescent Monomer

[0054] To a solution of 1-pyrene methanol (5 g, 21.5 mmol) in THF (150 mL) was added NaH (2g, 50 mmol). After stirring for 30 min., cinnamyl chloride (4.3 g, 28.7 mmol) was added drop wise. After 2.5 h of reflux, the reaction was quenched with water and the two layers separated. The organic solution was washed with water (2 X 100 mL) followed by washing with brine (2X 100 mL), dried over MgSO₄ and concentrated to an orange oil. The product was purified by column chromatography (1:1, CH₂Cl₂:hexanes) to yield 5 g (67% yield). The structure was confirmed by ¹H NMR analysis.

Example 2: Preparation of Fluorescent Nano Micelle Particles (FNMPs) with *t*-Butylstyrene

[0055] To a 10 oz. nitrogen purged bottle, cyclohexane (20 mL), *t*-butylstyrene (1.2 mL), oligomeric oxolanyl propane (OOPs) (0.03 mL, 1.6M) and butyl lithium (0.1 mL, 1.54M) were added. The bottle was placed into 80°C water bath for 10 minutes. After cooling to 23°C, a charge of cinnamyl-O-CH₂-pyrene (10 mL, 0.14M in cyclohexane) was added into the bottle. After continual cooling for 5 minutes, a charge of DVB (0.5 mL) was added to the mixture. The reaction proceeded for 1 hour, and then was then terminated by adding isopropanol (0.1 mL).

Example 3: Preparation of FNMPs with *t*-Butylstyrene

[0056] To a 10 oz. nitrogen purged bottle, hexane (20 mL), *t*-butylstyrene (1.2 mL), and butyl lithium (0.1 mL, 1.54M) were added. Then, the bottle was placed into an 80°C water bath for 30 minutes. The bottle was then cooled and maintained at a temperature of 23°C. A mixture of cinnamyl-O-CH₂-pyrene (10 mL, 0.14M in cyclohexane), DVB (0.5 mL) and *t*-butylstyrene (1 mL) was added to the bottle. The reaction proceeded at 23°C for 2 hours, and was then terminated by adding isopropanol (0.1 mL).

Example 4: Preparation of FNMPs with *t*-Butylstyrene

[0057] To a 10 oz. nitrogen purged bottle, hexane (20 mL), *t*-butylstyrene (1.2 mL), and butyl lithium (0.1 mL, 1.54M) were added. Then, the bottle was placed into 80°C water bath for 30 minutes and then cooled to 25°C. A mixture of cinnamyl-O-CH₂-pyrene (10 mL, 0.14M in cyclohexane), DVB (0.5 mL), and *t*-butylstyrene (1 mL) was added to the bottle. After the reaction proceeded at 23°C for 1 hour, *t*-butylstyrene (1 mL) was added to the bottle. After an additional 60 minutes, the reaction was terminated by adding isopropanol (0.5 mL).

Example 5 (Prospective): Preparation of FNMPs with Butadiene

[0058] To a 10 oz. nitrogen purged bottle, hexane (20 mL), butadiene (5 gr, 20% in hexane), oligomeric oxolanyl propane (OOPs) (0.03 mL, 1.6M solution) and butyl lithium (0.1 mL, 1.54M) would be added. The bottle would then be placed into 80°C

water bath for 10 minutes. After cooling to 23°C, a charge of cinnamyl-1-methylpyrene ether (10 mL, 0.14M in cyclohexane) would be added into the bottle. After continual cooling for 5 minutes, a charge of DVB (0.5 mL) would be added to the mixture. The reaction would proceed for 1 hour, and then would be terminated by adding isopropanol (0.1 mL).

Example 6 (Prospective): Preparation of FNMPs with Styrene Butadiene

[0059] To a 10 oz. nitrogen purged bottle, hexane (20 mL), styrene (1 gr, 30% in hexane), butadiene (5 gr, 20% in hexane), oligomeric oxolanyl propane (OOPs) (0.03 mL, 1.6M solution) and butyl lithium (0.1 mL, 1.54M) would be added. The bottle would be placed into 80°C water bath for 10 minutes. After cooling to 23°C, a charge of cinnamyl-1-methylpyrene ether (10 mL, 0.14M in cyclohexane) would be added into the bottle. After continual cooling for 5 minutes, a charge of DVB (0.5 mL) would be added to the mixture. The reaction would proceed for 1 hour, and then would be terminated by adding isopropanol 0.1 mL).

Example 7: Characterization of Fluorescent Nano Micelle Particles (FNMPs)

[0060] A 1 mL portion of the Example 3 solution was diluted to about a 1×10^{-4} wt% solution in toluene. A drop of the diluted solution was then coated on a graphed copper micro-screen. After the solvent evaporated, the screen was exposed to RuO₄ for about 5 minutes, and then examined by TEM. The image (see Figure 2) shows that the FNMPs have a mean size of about 40 nm.

Example 8: Characterization of Fluorescent Nano Micelle Particles (FNMPs)

[0061] A 5 mL aliquot was taken from the Example 3 reaction and added to an aluminum pan. After the solvent evaporated, a film of about 0.1 mm thickness resulted. The characterization was performed using an Olympus-BH2 microscope equipped with a Polaroid camera. The polymer film was examined under a UV light. The film showed fluorescence under green light (~ 450 to 510 nm). As shown in Figure 2, the film was entirely glowing as compared to the background. The experiment indicated that the desired nano-sized materials with fluorescent properties were produced.

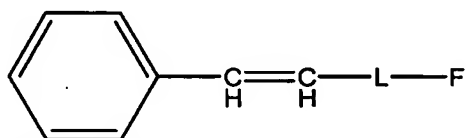
[0062] While the invention has been illustrated and described by way of examples, it is not intended to be limited to the details shown, since various modifications and substitutions can be made without departing in any way from the spirit of the present invention. As such, further modifications and equivalents of the invention herein disclosed may occur to persons skilled in the art using no more than routine experimentation, and all such modifications and equivalents are believed to be within the spirit and scope of the invention as defined by the following claims.

It is claimed:

1. A fluorescent nanoparticle comprising:

- (a) a core made from at least one alkenylbenzene monomers;
- (b) an intermediate layer comprising polymer chains;
- (c) a shell layer comprising an outer surface of the nanoparticle;
- and
- (d) at least one fluorescent portion;

the fluorescent portion arising from the addition of a corresponding monomer with a structure represented by the following formula:



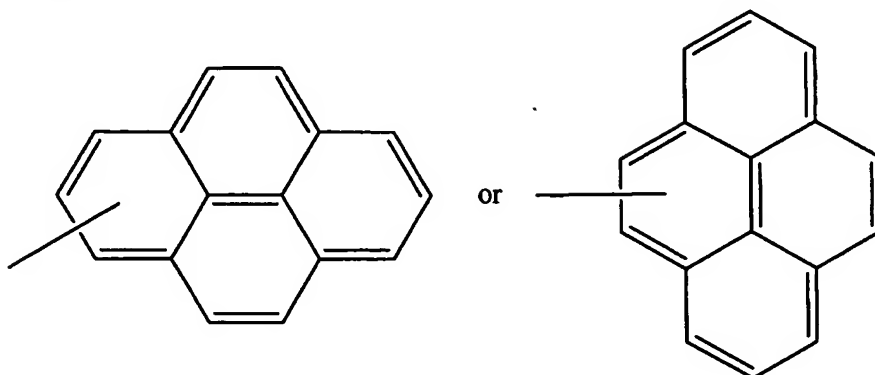
wherein L is a direct bond or a divalent group, and F is any fluorescent moiety;

wherein the fluorescent portion is located in at least one of the following locations: the core, the intermediate layer, or the shell layer of the nanoparticle.

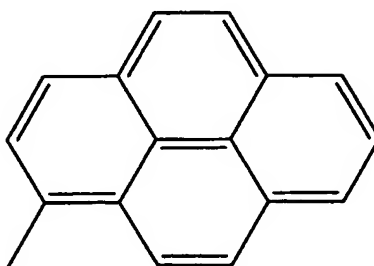
2. The fluorescent nanoparticle of claim 1, wherein the polymer chains are selected from the group consisting of: polystyrene, polybutadiene, polyisoprene, copolymers of a combination of styrene, butadiene, or isoprene, derivatives thereof, or mixtures thereof.

3. The fluorescent nanoparticle according to claim 1, in which the fluorescent moiety F is selected from the group consisting of pyrene, perylene, phenanthrene, anthracene, naphthalene, and combinations thereof.

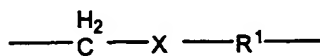
4. The fluorescent nanoparticle according to claim 1, in which the fluorescent moiety F has formula of:



5. The fluorescent nanoparticle of claim 1, in which the fluorescent moiety F has a formula of:

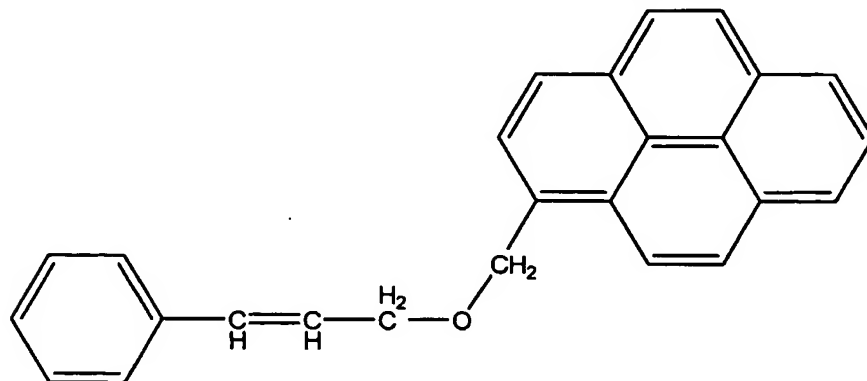


6. The fluorescent nanoparticle of claim 1, in which the linker group L is:

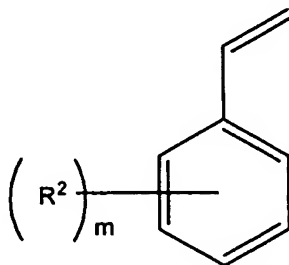


wherein X comprises a heteroatom; and R¹ is a straight or branched C₁-C₈ alkylene group.

7. The fluorescent nanoparticle of claim 1, in which the at least one fluorescent portion contains one or more monomers with a formula of:

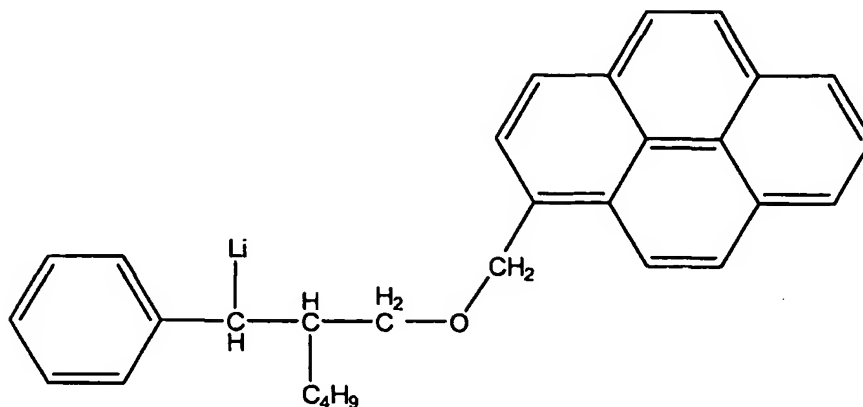


8. The fluorescent nanoparticle of claim 1, in which the at least one alkenylbenzene monomer has a formula of:

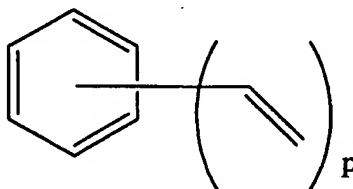


wherein m is an integer and $1 \leq m \leq 5$; and R^2 is selected from saturated or unsaturated, substituted or unsubstituted, straight or branched, cyclic or acyclic C_3 - C_8 alkyl groups.

9. The fluorescent nanoparticle of claim 11, in which the fluorescent initiator has a formula of:

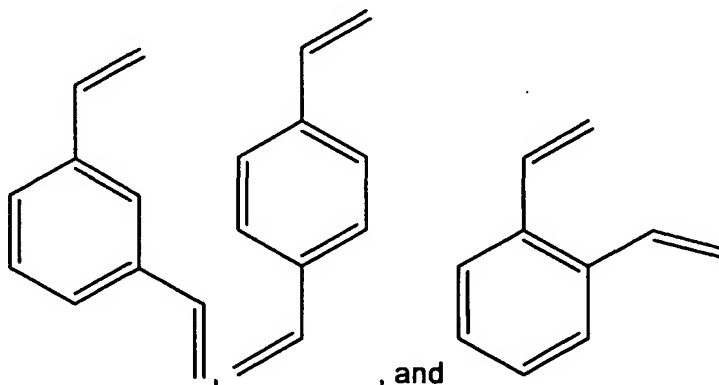


10. The fluorescent nanoparticle of claim 1, wherein the core is crosslinked using a multiple vinyl-substituted aromatic hydrocarbon having a formula of:



wherein p is an integer and $2 \leq p \leq 6$.

10. The fluorescent nanoparticle of claim 13, in which the multiple vinyl-substituted aromatic hydrocarbon is selected from one of the following isomers or any combination thereof:



12. The fluorescent nanoparticle of claim 1, which has a generally spherical shape with a mean average diameter of less than about 100 nm.
13. A method for preparing a fluorescent nanoparticle, the method comprising:
- (i) preparing a living polymer with a fluorescent portion by a step selected from the group consisting of:
 - (a) copolymerizing a fluorescent monomer with a non-fluorescent monomer;
 - (b) polymerizing a monomer with a fluorescent initiator; and
 - (c) polymerizing a monomer to produce a living polymer, and subsequently adding a fluorescent monomer to the living polymer;
 - (ii) adding a crosslinking agent;
 - (iii) quenching the living polymer chain ends with a proton source.
14. The method of claim 13, wherein after step (i) the living polymer with a fluorescent portion self-assembles into a micelle in a liquid hydrocarbon medium.
15. The method of claim 14, in which the liquid hydrocarbon medium comprises pentane, isopentane, 2,2 dimethyl-butane, hexane, heptane, octane, nonane, decane, cyclopentane, methyl cyclopentane, cyclohexane, methyl cyclopentane, cycloheptane, cyclooctane, cyclononane, cyclodecane, or a combination thereof.
16. The method of claim 13, wherein the living polymer with the fluorescent portion is prepared by polymerizing an alkenylbenzene to produce a polyalkenylbenzene, and subsequently adding a fluorescent monomer to the polyalkenylbenzene after or at the same time that the crosslinking agent is added.
17. The method of claim 13, further comprising the step of copolymerizing the living polymer with a monomer.
18. A fluorescent nanoparticle comprising:

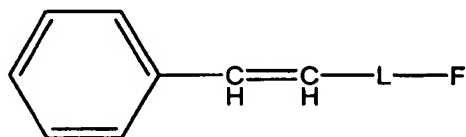
a core, an intermediate layer, and a shell layer;

the intermediate and shell layers include polymer chains extending from the intermediate layer into the shell layer, the shell being the outermost layer of the nanoparticle;

the core including alkenylbenzene monomers, the alkenylbenzene monomers having the same structure or being a mixture of two or more different structures; the alkenylbenzene monomers crosslinking living polymer chain ends of the polymer chains of the intermediate layer;

a fluorescent portion located in at least one of the intermediate or shell layers, or at the core;

the fluorescent portion including at least one fluorescent monomer with a structure represented by the following formula:



wherein L is a direct bond or a divalent group, and F is a fluorescent moiety.

19. The fluorescent nanoparticle of claim 18, wherein blocks of the polymer chains in the intermediate and shell layers are more soluble in a hydrocarbon solvent than blocks of the polymer chains in the intermediate layer that are nearer the core.

20. The fluorescent nanoparticle of claim 18, wherein the nanoparticle has a diameter of less than about 200 nm.

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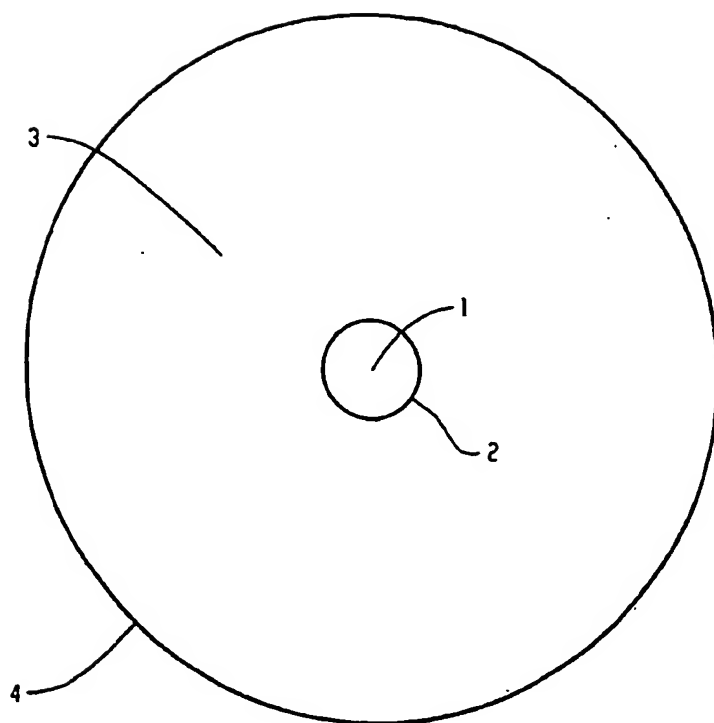


Fig. 1

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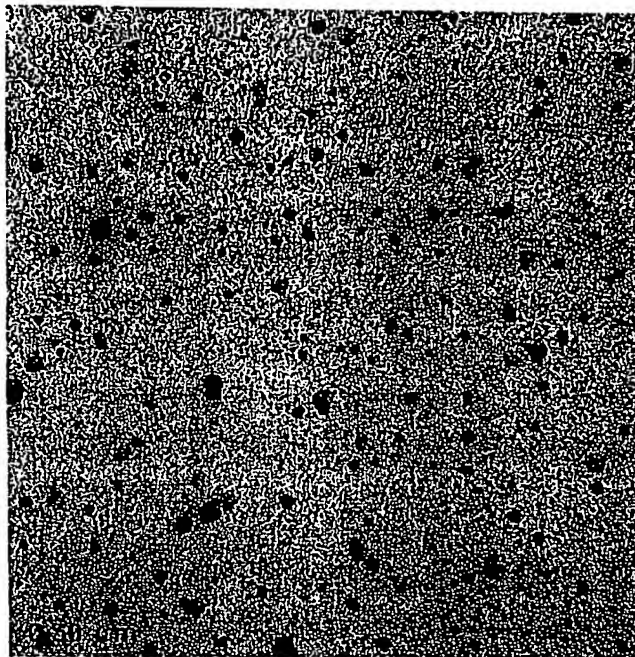


Fig. 2

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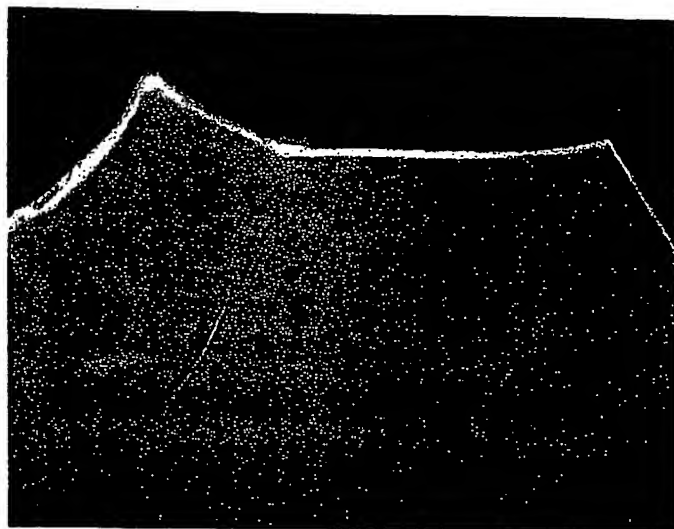


Fig. 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/026031

A. CLASSIFICATION OF SUBJECT MATTER
INV. C08F297/02 G01N33/58

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C08F G01N.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data, COMPENDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>O'REILLY RACHEL K ET AL: "Functionalization of micelles and shell cross-linked nanoparticles using click chemistry" CHEM. MATER.; CHEMISTRY OF MATERIALS NOV 29 2005, vol. 17, no. 24, 29 November 2005 (2005-11-29), pages 5976-5988, XP002480353 abstract. page 5976, right-hand column, line 3 - line 7 page 5977, left-hand column, line 8 - line 11 page 5978, left-hand column, line 7 - right-hand column, line 6 page 5980, left-hand column, line 14 - line 28 page 5980, right-hand column, line 17 - -/--</p>	<p>1-12, 18-20</p>

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search

16 May 2008

Date of mailing of the international search report

30/05/2008

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Authorized officer

Iraegui Retolaza, E

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/026031

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	<p>line 21 page 5981, right-hand column, line 28 - line 34 page 5982, right-hand column; line 16 - left-hand column, line 11 page 5983, right-hand column, line 13 - page 5985, left-hand column, line 5 page 5985, left-hand column, line 17 - line 28 page 5985, right-hand column, line 5 - line 8 page 5987, left-hand column, line 1 - line 11 page 5987, right-hand column, line 27 - page 5988, left-hand column, line 13</p>	
A,P	<p>EP 1 783 168 A (FUJIFILM CORP [JP]) 9 May 2007 (2007-05-09) paragraphs [0144], [0159] - [0161]; claim 15</p>	1-20
A	<p>FR 2 099 645 A (XEROX CORP XEROX CORP [US]) 17 March 1972 (1972-03-17) page 2, line 3 - line 9 page 3, line 35 - page 4, line 14; claims; examples</p>	1-20

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Information on patent family members

International application No

PCT/US2007/026031

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